The association of the *ACTN3* R577X and *ACE* I/D polymorphisms with athlete status in football: a systematic review and meta-analysis

Alexander B. T. McAuley[1], David C. Hughes[1], Loukia G. Tsaprouni[1], Ian Varley[2], Bruce Suraci[3], Thomas R. Roos[4], Adam J. Herbert[1], and Adam L. Kelly[1]

[1]Faculty of Health, Education and Life Sciences, Birmingham City University, Birmingham, West Midlands, United Kingdom; [2]Department of Sport Science, Nottingham Trent University, Nottingham, United Kingdom; [3]Academy Coaching Department, AFC Bournemouth, Bournemouth, United Kingdom; [4]The International Academy of Sports Science and Technology (AISTS), University of Lausanne, Lausanne, Switzerland

Correspondence: A. McAuley, Department of Life Sciences, Birmingham City University, City South Campus, Westbourne Road, Edgbaston, B15 3TN, UK. E-mail: Alex.Mcauley@mail.bcu.ac.uk

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The aim of this review was to assess the association of *ACTN3* R577X and *ACE* I/D polymorphisms with athlete status in football and determine which allele and/or genotypes are most likely to influence this phenotype via a meta-analysis. A comprehensive search identified 17 *ACTN3* and 19 *ACE* studies. Significant associations were shown between presence of the *ACTN3* R allele and professional footballer status (OR = 1.35, 95% CI: 1.18-1.53) and the *ACE* D allele and youth footballers (OR = 1.18, 95% CI: 1.01-1.38) compared to a control group. More specifically, the *ACTN3* RR genotype (OR = 1.48, 95% CI: 1.23-1.77) and *ACE* DD genotype (OR = 1.29, 95% CI: 1.02-1.63) exhibited the strongest associations, respectively. These findings may be explained by the association of the *ACTN3* RR genotype and *ACE* DD genotype with power-orientated phenotypes and the relative contribution of power-orientated phenotypes to success in football. As such, the results of this review provide further evidence that individual genetic variation may contribute towards athlete status and can differentiate athletes of different competitive playing statuses in a homogenous team-sport cohort. Moreover, the *ACTN3* R577X and *ACE* I/D polymorphisms are likely (albeit relatively minor) contributing factors that influence athlete status in football.

Keywords: Soccer; Team-Sport; Genetics; SNP; Genomics.

1 Introduction

Actinin alpha 3 (ACTN3), a member of the actin family, is a sarcomeric protein which is greatly expressed in muscle tissue [1]. A function of the ACTN3 protein involves crosslinking fast-twitch (type II) actin filaments in skeletal muscle fibres [2]. Thus, the expression of the ACTN3 protein in glycolytic skeletal muscle is thought to be a contributing factor to the generation of powerful and explosive muscle contractions; through optimal coordination of type II muscle fibres [3]. The coding of the ACTN3 protein is controlled by the *ACTN3* gene, located on chromosome 11q13.2. A common genetic variant in the *ACTN3* gene has been identified which significantly alters the production of the ACTN3 protein [4]. The genetic variation is a nonsense single nucleotide polymorphism (SNP) which can introduce a premature stop codon within the gene at position 577 (rs1815739) [5]. Cytosine is the most common nucleotide at this position (i.e., **C**GA), which encodes the amino acid, arginine (R) [2]. Alternatively, thymine can be possessed by an individual (i.e., **T**GA), producing the stop codon (X); potentially resulting in an individual being deficient in ACTN3 [4]. As the *ACTN3* gene portrays a role in force production, it has been hypothesised that the performance of activities requiring extensive force production (i.e., sprinting, jumping, weightlifting) would be influenced by whether an individual possesses the R allele or RR genotype. Many studies have reported that either the RR genotype was over-represented, or the XX genotype was underrepresented, in power-related sports (e.g., 100m sprint, rowing, speed skating, artistic gymnastics, sprint swimming, Olympic weightlifting) across American, Polish, Finnish, Italian, Japanese, Israeli, and Russian cohorts [6–15]. Indeed, Ma and colleagues [16] conducted a meta-analysis on 23 studies involving power and endurance athletes and discovered that the R allele was only associated with power athletes. Moreover, a recent meta-analysis on solely power athletes reported similar associations between the R allele and power athletes across 38 studies [17].

Another commonly investigated gene in sport performance is the angiotensin I converting enzyme (*ACE*) gene. The angiotensin I converting enzyme catalyses the degradation of the inactive decapeptide angiotensin I, and subsequently generates the physiologically active peptide, angiotensin II; an oligopeptide of eight amino acids that binds to specific receptors in the body affecting several systems [18,19]. Angiotensin II can constrict blood vessels and stimulate aldosterone production, resulting in increased blood pressure, thirst, or the dire for salt. As such, the ACE enzyme is the most crucial component of the renin-angiotensin system (RAS), as it is a potent vasopressor and aldosterone-stimulating peptide which regulates blood pressure and fluid-electrolyte balance [20]. A polymorphism has been identified within intron 16 of the *ACE* gene, located on chromosome 17q23.3 (NC_000017.11), which results in a substantial variation of RAS activity [21,22]. The polymorphism is known as an insertion/deletion (indel) polymorphism, with the insertion (I allele) and deletion (D allele) representing the presence and absence of a 287-bp Alu-sequence respectively. Specifically, the I allele has been associated with lower serum and tissue ACE activity, alongside an increased percentage of slow-twitch (type I) muscle fibres; whilst the D allele has been associated with higher circulating and

tissue ACE activity, alongside greater strength and muscle volume and an increased percentage of type II muscle fibres [21–23]. In the context of sport, the I allele has been frequently associated with elite endurance performance. Specifically, higher I allele frequencies have been reported in middle- and long-distance rowers, swimmers, road-cyclists, runners, mountaineers, cross-country skiers, and tri-athletes across a range of diverse cohorts (e.g., British, Australian, Croatian, Russian, Spanish, Italian, Turkish, Polish, Japanese, Indian) [24–33]. Indeed, during the meta-analysis of Ma and colleagues [16], the authors also assessed the influence of the *ACE* I/D polymorphism on endurance athletes over 25 studies, reporting that the II genotype was significantly associated with endurance athletes. However, the authors found no association between the *ACE* I/D polymorphism and power athletes. This may have been due to the large heterogeneity observed between studies (I^2 = >75%), most likely a result of not analysing the power athletes independently based upon ethnicity. Indeed, a more recent meta-analysis did conduct an ethnic specific analysis of power athletes and reported significant associations with the *ACE* D allele [34].

The collective results on the associations of the *ACTN3* R577X and *ACE* I/D polymorphisms, with athletic performance, complicate the implications for sports which require both power and endurance related traits, such as football. Football is an intermittent sport which requires optimal utilisation of both the aerobic and anaerobic systems [35,36,37,38]; and as such, predicting whether a power or endurance-orientated allelic variant may be preferable is not straightforward. Therefore, genetic association studies began to investigate whether a power or endurance-orientated genotype was more important in football by analysing polymorphisms such as *ACTN3* R577X and *ACE* I/D [39–41]. Moreover, studies began to assess if there was a difference between football players of various competitive playing levels (i.e., elite, non-elite; professional [PRO], non-professional [NP]) and controls (CON), in order to determine if *ACTN3* R577X or *ACE* I/D were associated with athlete status [42–44]. Currently, there is no general consensus on the importance of *ACTN3* or *ACE* in footballers, with studies reporting positive, negative, and contrasting allelic associations [39–44]. This is most likely because each gene or genotype has a small contribution to sporting performance and is dependent on numerous inter-individual variations (e.g., ethnicity and competitive playing level) [45,46]. As a result, studies require large homogenous sample sizes in order to have sufficient statistical power and demonstrate significant associations and replications [45,47]. However, studies within football genomics have notoriously small sample sizes and many are heterogenic multi-sport studies; mainly due to the unique population and limited access available [48]. Therefore, to overcome the limitation of sample sizes and heterogeneity, a meta-analysis can be used to pool the results of single homogenous studies together [47]. As such, the aim of this study was to assess if the *ACTN3* R577X and/or *ACE* I/D polymorphism are associated with athlete status in football by conducting a systematic review and meta-analysis.

2 Methodology

2.1 Search Strategy and Inclusion/Exclusion Criteria

In accordance to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines [49], the following search strategy was implemented. A comprehensive search of the Pubmed, SPORTDiscus, and MEDLINE databases was conducted on March 3rd 2020. For *ACTN3* the following Boolean search was used: ((football) OR (soccer)) AND (actn3) OR (alpha-actinin-3) OR (actinin-alpha-3) OR (R577X) OR (rs1815739)). For *ACE* the following Boolean search was used: ((football) OR (soccer)) AND (ace) OR (angiotensin I converting enzyme) OR (rs1799752) OR (rs13447447) OR (rs4341) OR (rs4646994)). Additionally, Google Scholar was searched using word combinations of the aforementioned Boolean searches, with no year restriction placed on any search. Furthermore, reference lists of the identified articles were searched for additional relevant studies. At the initial screening stage studies were included if they: (1) were primary cohort or case-control investigations; (2) presented *ACTN3* R577X or *ACE* I/D genotype frequencies of footballers in isolation; and (3) were published in the English language. Therefore, studies were excluded if they: (1) were reviews; (2) presented *ACTN3* R577X or *ACE* I/D genotype frequencies of footballers combined with other sports; and (3) were published in a language other than in English.

2.2 Data Extraction and Analysis

We extracted the following data from all studies: first author's name and year of publication; number of footballers and CON; nationality and ethnicity; gender; age range; competitive playing level; type of study (cohort or case-control); and distribution of genotype frequencies in footballers and CON. Extracted data was then analysed in the following order: 1) pooled genotype frequencies of case-control studies in isolation; 2) pooled genotype frequencies of case-control studies combined with cohort studies (with an ethnically matched independent CON population added); and 3) sub-group analysis of ethnicity, gender, and level of competition. Independent CONs were added to cohort studies from the 1000 genomes database (https://www.internationalgenome.org) or an independent non-included study. Each cohort study was assigned a CON which was not used by any other study in the analysis to bolster the number of unique individuals and decrease selection bias. All of the included CON were also subjected to risk of bias before being included.

2.3 Risk of Bias

After initial primary inclusion, all studies were subjected to Hardy–Weinberg equilibrium (HWE) via chi-square (significance level $P<0.05$); culminating in the removal of all studies violating this significance threshold, as deviations from HWE in a CON group can indicate potential genotyping errors, selection bias and stratification [50]. Furthermore, as each study is tested for HWE, Benjamini-Hochberg false discovery rate (FDR) is used to correct p-values [51]. Additionally, as this review only uses published studies, publication bias could be a limiting factor. Therefore, Egger's test [52] in

combination with funnel plots [53] were used to identify if publication bias was present and potentially skewing results (significance level P<0.05).

2.4 Statistical Analysis

To measure the strength of the association between the *ACTN3* R577X and *ACE* I/D polymorphisms and football players, odds ratios (OR), 95% confidence intervals (CI), and forest plots were used via either a fixed-effects model or random-effects model to identify the individual and pooled effects of the studies. Determining which model was appropriate for each analysis was based on the level of heterogeneity revealed via the I^2 (<50% = fixed-effects model; >50% = random-effects model) and Cochran's Q [54] statistical test (significance level at P<0.05). Four genetic models were used to assess genotype and allele differences between football players and CONs: (a) allele contrast; (b) recessive; (c) dominant; and, (d) over-dominant. Additionally, pair-wise comparisons were also conducted. Finally, a sensitivity analysis was conducted via a leave-one-out approach in order to test the robustness of results [55]. All analyses were conducted using the MetaGenyo online Statistical Analysis System software (http://bioinfo.genyo.es/metagenyo/) [56].

3 Results

3.1 Search Process

The systematic search processes initially identified 588 studies (*ACTN3*, n = 290; *ACE*, n = 298). Following the removal of duplicates and the screening of titles and abstracts, full-text assessment commenced; culminating in 17 *ACTN3* and 19 *ACE* studies being judged as adequately meeting the predetermined inclusion criteria and subsequently being included in the final analysis (see Figure 1 for full systematic search process).

****Insert **Figure 1.** near here****

3.2 Study Characteristics

The 17 *ACTN3* studies consisted of nine case-control and eight cohort studies respectively. There were a total of 1759 football players included across all studies (aged 10-37 years), with sample sizes ranging from 25-353. The most frequently studied nationality was Brazilian (n = 850), whilst the most frequently studied ethnicity was Caucasian (n = 587). Eleven studies included PRO players (n = 713; aged 17-37 years); four studies included NP players (n = 447; aged 14-30 years); and, two included both PRO and NP players (n = 599; aged 10-27 years). The 19 *ACE* studies consisted of 15 case-control and four cohort studies. There were a total of 1925 football players included across all studies (aged 10-27 years), with sample sizes ranging from 25-353. The most frequently studied nationality was Brazilian (n = 709), whilst the most frequently studied ethnicity was Caucasian (n = 802). Ten studies included PRO players (n = 674; aged 17-26 years); seven studies included NP players (n = 652; aged 15-21 years); and, two included both PRO and NP players (n = 599; aged 10-27 years).

3.3 Risk of Bias

Fifteen of the originally included 17 *ACTN3* studies remained after risk of bias assessment. The studies excluded, with reasons for exclusion, were as follows: (1) Massidda et al. [57] failed HWE assessment; and, (2) La Montagna et al. [58] failed to provide the specific nationality and ethnicity of the footballers, thus an ethnically-matched CON could not be added. Seventeen of the originally included 19 *ACE* studies remained after risk of bias assessment. The following studies were excluded due to failing HWE assessment: Gineviciene et al. [59] and Galeandro et al. [41]. In addition, the Bulgarian sub-cohort of Andreeva et al. [60] was excluded from analysis due to also failing HWE assessment. In all genetic comparison models that identified a significant association with *ACTN3* and *ACE*, funnel plots did not reveal any signs of asymmetry and Egger's test did not detect a significant indication of publication bias.

3.4 Main Analysis

Several significant associations were observed between the *ACTN3* R577X polymorphism and genetic comparison models with case-control studies in isolation: (1) allele contrast; (2) recessive; (3) dominant; (4) RR vs. XX; and, (5) RR vs. RX. In addition, similar associations with the same genetic models were also observed in case-control studies combined with cohort studies; with the only exception being the additional significant association of RX vs. XX. The strongest association observed in both case-controls in isolation and combined with cohorts was RR vs. XX (see Table 1 for ORs and CIs of each *ACTN3* comparison). Sensitivity analysis assessed the robustness of the results and revealed that no study significantly altered pooled ORs. Conversely, no significant associations were observed between the *ACE* I/D polymorphism with case-controls in isolation or combined with cohorts using any genetic comparison model (see Table 2 for ORs and CIs of each *ACE* comparison).

3.4.1 Sub Analyses

The first sub-analysis assessed the independent associations of PRO players and NP players vs. controls (CON). Several significant associations were observed between the *ACTN3* R577X polymorphism and genetic comparison models in PROs: (1) allele contrast; (2) recessive; (3) dominant; (4) RR vs. XX; and, (5) RR vs. RX. The strongest association observed was RR vs. XX. No significant associations were observed between the *ACTN3* R577X polymorphism and genetic comparison models in NPs vs. CON. Conversely, no significant associations were observed between the *ACE* I/D polymorphism and PRO players. However, several significant associations were observed between the *ACE* I/D polymorphism and NP players: (1) allele contrast; (2) dominant; and, (3) ID vs. DD.

The second sub-analysis assessed the independent influence of ethnicity and nationality on associations. Due to the variance in geographical ancestry, Caucasian and Brazilian were the only ethnicity and nationality eligible for analysis. Several significant associations were observed between the *ACTN3* R577X polymorphism and genetic comparison models in Caucasians: (1) allele contrast;

(2) recessive; (3) dominant; and, (4) RR vs. XX. Likewise, several significant associations were also observed between the *ACTN3* R577X polymorphism and genetic comparison models in Brazilians: (1) allele contrast; (2) dominant; (3) RR vs. XX; and, (4) RX vs. XX. The strongest association observed in both Caucasians and Brazilians was RR vs. XX. Conversely, no significant associations were observed between the *ACE* I/D polymorphism and Caucasians or Brazilians.

****Insert **Table 1.** near here****

****Insert **Table 2.** near here****

4 Discussion

The aim of this study was to assess if the *ACTN3* R577X and/or *ACE* I/D polymorphism were associated with athlete status in football; and if so, determine which allele and/or genotypes are most likely to influence this phenotype via a meta-analysis. To the author's knowledge this is the first review to do this within football; and moreover, it is the first meta-analysis in a homogenous team-sport cohort.

4.1 Main Analysis

Following meta-analysis, this review identified several associations between the *ACTN3* R577X polymorphism and all football players (PRO & NP combined) vs. CON. In summary, the main associations were observed in football players possessing the R allele, with the strongest association being the RR vs. XX genotype. These associations were observed in case-control studies in isolation and remained similar with the addition of cohort studies. Although, with the significant increase in sample size from cohort studies the CI was reduced, possibly indicating a more accurate estimation. The only notable difference between genetic comparison models was that the RX vs. XX pair-wise comparison was statistically significant with cohorts added vs. non-significant in case-controls in isolation. Therefore, only the over-dominant model remained non-significant in both case-control studies in isolation and with the addition of cohort studies; most likely due to the strength of the association of the RR genotype. As such, the results of this analysis showcase that in football players, similar to power athletes, there is an overrepresentation of the *ACTN3* RR genotype. For instance, this can be illustrated by the similar recessive model and dominant model findings of the present study and Ma and colleagues [16], respectively. This can likely be explained by the combination of several factors: (1) the number and frequency of powerful actions performed in a game (i.e., 1000-1400 acyclical bursts of activity, including; jumps, tackles, shots at goal, changes of direction, and, sprints), with a high-intensity sprint occurring every ~70s [61,62]; (2) the contribution of the ability to repeat higher intensity actions to success in football (i.e., league position, goals scored, goals prevented, duel success) [63,64]; and, (3) the association of the RR genotype with power-orientated phenotypes (i.e., vertical jumping and 10-30m sprints) [65,66]. However, it is important to recognise that there are many other genetic polymorphisms, with each likely influencing performance to a limited extent [45,46]. Therefore, football

players, power athletes, and indeed other team-sports, may possess contrasting allele and genotype frequencies in other polymorphisms. Hence, the results of this review only indicate a difference between football players and non-athletic CONs. However, this difference appears to be greatly mediated by competitive playing level, which may have contributed to the heterogeneity present between *ACE* studies; and as a result, the non-significant associations between *ACE* and PRO & NP players combined. Indeed, the results of the subsequent sub-analysis on competitive playing level revealed that *ACE* may play an important role in determining athlete status earlier in athlete development. Therefore, although *ACE* was not associated with athlete status in the main analysis, it is still an important genetic variant in football, depending on specific competitive playing levels.

4.2 Sub-Analyses

The importance of the competitive playing level of footballers in this review was assessed via separating PRO players and NP players. PRO players were classified as players that studies specifically described as playing at a professional level, whereas NP players were classified as players playing at a semi-professional, amateur, or youth level. This particular method of categorisation was chosen, as opposed to elite vs. non-elite, to circumvent the problematic classification issue of 'eliteness' (i.e., what constitutes elite status and how do we define it?) [67,68]. For example, players may be internationals who represent their country (normally classed as elite), however what if their country is near the bottom of the international rankings? Furthermore, players may play for a European club positioned 1st in their country's highest league (normally classed as elite), but what if the country and club both have a low UEFA coefficient ranking? Moreover, how do we define the highest performing youth players? Given that no irrefutable solution has been provided and no general consensus has been agreed, the term 'elite' is still inconsistently utilised in the literature to describe varying standards of performance [67,68]. Therefore, to reduce between-study-heterogeneity, the authors chose to compare PROs and NPs (limitations of this approach are discussed in section 4.3).

Firstly, the results of this analysis revealed that the *ACTN3* R577X polymorphism was associated with PRO players vs. CON. However, no statistically significant difference was observed between NP players and CON. Moreover, the strength of the association between the R allele, and specifically the RR genotype, was even stronger in PRO players when separated from NP players. As such, these results indicate that the R allele is a likely (albeit small) contributing factor towards attaining PRO status in football. Secondly, whilst the *ACE* I/D polymorphism was not associated with PRO players vs. CON, it was associated with NP players vs. CON. To be specific, NP players were more likely to possess a D allele or DD genotype. This is perhaps more easily demonstrated via inverse statistical analysis: (1) D vs. I (OR = 1.18, 95% CI: 1.01-1.38); (2) DD vs. DI+II (OR = 1.29, CI: 1.02-1.63); and, (3) DD vs. DI (OR = 1.32, CI: 1.03-1.69). Interestingly, the NP players consisted solely of youth players in the *ACE* I/D studies (n = 652; aged 15-21 years) showcasing that the D allele is only overrepresented specifically in youth football. Furthermore, the differences between PROs and NPs

may have increased further given that Egorova and colleagues [42] was not included in this analysis, due to not displaying the individual genotype frequencies of PRO and NP players in their study. However, the authors did report that when separated by competitive playing level, only elite players displayed a significantly higher frequency of the *ACTN3* R allele compared to CON (81.1%, $P < 0.001$); whilst conversely, only youth players displayed a significantly higher frequency of the *ACE* D allele compared to CON (78.8%, $P < 0.001$). As such, the observed cumulative evidence suggests that whilst possessing the *ACE* D allele is potentially more beneficial to young players, the *ACTN3* R allele is the only allele which likely has a (minor) role in attaining PRO status (see Figure 2). However, it is important to note the use of terms such as "albeit small" and "minor" when interpreting these findings. Polymorphisms account for very little of the inter-individual variance in complex traits such as athlete status [45]. Indeed, even a combination of 97 polymorphisms (at $P = 5\times10^{-8}$ or better) could only account for 2.7% of body mass index variance between individuals[69]. Therefore, as evidenced by the relatively small odds ratios in this study, *ACTN3* and *ACE* similarly play a minor role in determining athlete status in football.

****Insert **Figure 2.** near here****

The explanation for the observed associations between the *ACE* D allele and *ACTN3* R allele with youth and PRO players respectively is challenging. Both alleles have been previously associated with strength/power orientated sports and general strength/power characteristics (i.e., increased percentage of type II muscle fibres; strength; and, muscle mass) [13,23]. More specifically, in football cohorts both alleles have been positively associated with greater countermovement and squat jump performance, and faster 10m, 20m, and 30m sprint times [65,66]. As such, both alleles appear to be associated with the same side of the endurance-power continuum in football. Therefore, it is interesting that each allele is independently associated with different competitive playing levels. However, perhaps the categorisation method employed to distinguish competitive playing levels in this review could possibly be responsible. For example, whilst heterogeneity between studies in the PRO vs. NP analysis was mostly small, the age of NPs in *ACTN3* ranged from 14-30 years; whereas the age of NP players in *ACE* ranged from 15-21 years. As such, it is possible that the NP *ACE* players had greater performance levels than the NP *ACTN3* players, as the NP *ACE* players may play at the highest youth level in their respective age groups; whilst the *ACTN3* players may not. Indeed, several previous studies have demonstrated that 'elite' youth players, aged 14-17 years, have outperformed non-elite players in acceleration, speed and jumping assessments [70,71]. Although, it could be argued that grouping young players (14-17 years) as elite and non-elite is not appropriate as factors such as maturation status may influence performance more than *ACTN3* or *ACE* genotype. However, in truth, numerous potential explanations exist, including: (1) differences in competitive performance levels; (2) variations in geographical ancestry; (3) disparities in the distributions of players relative to their on-field position within samples; (4) distinct methods of genotyping; (5) individual gene-gene interactions; and, (6) separate gene-environment interactions. Moreover, it is also important to note that the *ACE* gene is part

of a very complex pathway, with serval interactions that can influence it's activity; whilst *ACTN3* represents the presence/absence of a structural protein [18,19]. As such, the specific cause of the distinction between the *ACTN3* R577X and *ACE* I/D polymorphisms with PRO and NP players requires further research to elucidate these findings.

In addition to competitive playing level, sub-analysis also assessed the influence of ethnicity and geographical ancestry on observed associations. Therefore, studies, and samples within studies, were separated based upon their reported ethnic heritage and nationality. After separation, Caucasians and Brazilians collectively represented 81% and 79% of *ACTN3* and *ACE* studies respectively. Therefore, only these could be assessed via comparison analysis. Firstly, in relation to the *ACE* I/D polymorphism, no significant associations were observed in either Caucasians or Brazilians. However, in relation to the *ACTN3* R577X polymorphism, several significant associations were observed in both Caucasians and Brazilians between the R allele and football players vs. CON. However, in models with significant associations in both Caucasians and Brazilians (allele contrast, dominant, RR vs. XX), Caucasians displayed higher ORs. This suggests the R allele, and more significantly the RR genotype, appear to be of greater importance to footballers of Caucasian heritage. This suggestion is bolstered by the significant associations observed solely between Caucasians and a recessive model, and between Brazilians and the pair-wise comparison of the RX vs. XX genotypes. This reveals that the RX genotype may be more associated with footballing status in Brazilians, whilst the RR genotype may be more associated with footballing status in Caucasians.

The possible cause of the disparities in ORs between the R allele in Brazilians and Caucasians may be related to the ethnic diversity of the Brazilian population; and more specifically, the proportion of individuals with African ancestry. Individuals of African ancestry have greater frequencies of the RR genotype (~78%) and significantly lesser frequencies of the XX genotype (~1%), irrespective of athlete status, compared to the estimated frequencies of the world population (RR ~40%; XX ~18%) [72–74]. As such, the *ACTN3* R577X polymorphism does not differentiate elite athletes from CON of African ethnicity in either power or endurance-orientated sports [72,73]. Using ancestry informative markers, previous studies have reported that the Brazilian population are formed of ~65% European SNPs, ~22% African SNPs, and ~13% Amerindian SNPs [75]. Furthermore, these proportions vary in each independent region of Brazil. For example, it is estimated that the population of Rio de Janeiro is formed of ~55% European SNPs, ~31% African SNPs, and ~14% Amerindian SNPs [76]. As such, given the genetic similarity of the Brazilian population with the genetic profile of African ethnicities, this potentially explains why the RR genotype may contribute to footballing status in Brazil to a lesser extent.

4.3 Strengths and Limitations

This study's findings are reinforced due to the small-moderate heterogeneity identified between studies. Only two of the 28 total measurements regarding *ACTN3* displayed an I^2 value over 50% and

violated Cochran's Q statistical threshold (P<0.05); although neither of these two measurements displayed a statistically significant result. Likewise, neither of the three associations identified between the *ACE* I/D polymorphism and NP players displayed significant heterogeneity. Furthermore, no suggestion of publication bias was identified regarding the observed significant associations, through analysis of funnel plots or Egger's test (P<0.05).

This review is not without limitations and each must be duly considered when interpreting the findings. Firstly, this review attempted to include both male and female football players, but unfortunately the authors could only locate one study involving female players [60] which met the pre-defined inclusion criteria. Therefore, the results from this review mainly apply to male football players. Secondly, the ethnic and geographic implications of this review mainly concern Brazilians and Caucasians, due to both representing the majority of the football players included in this review (*ACTN3* = 48% & 33% respectively; *ACE* = 37% & 42% respectively). Thus, research is still required on players of diverse geographical ancestry. Thirdly, the division of players into PRO and NP categories was chosen to circumvent the well-known issues surrounding what constitutes 'eliteness' in sport; however, the PRO level still encompasses substantial disparities in standards of play (e.g., English Premier League vs. English Football League Two). As such, this makes it difficult to objectively quantify player ability and consequently associate the findings with specific levels of performance. Finally, the on-field positions of footballers were rarely reported in the included studies. This is important considering the previously reported inter-positional associations between the *ACTN3* R577X and *ACE* I/D polymorphism and football players, such as forwards and goalkeepers possessing significantly higher frequencies of the *ACTN3* R allele and *ACE* D allele, respectively [42]. Therefore, it is unknown whether the associations in this review were influenced by the number of players included in each study relative to their on-field positions.

4.4 Practical Applications

Limited evidence exists supporting the practical application of genetic information with athlete development. For example, it has been reported that individuals possessing the *ACTN3* RR and *ACE* DD genotypes may have an improved adaptive response in strength and power with heavy resistance training [77,78]. As such, Kikuchi and Nakazato [79] suggested that individuals possessing power-orientated genetic variants, such as *ACTN3* RR, may benefit more from resistance training consisting of high weights with low-repetitions for strength and power. Jones and colleagues [80] investigated this theory and reported that in 39 football players the *ACE* DD and *ACTN3* RR genotypes demonstrated a greater improvement in countermovement jump performance in response to high-weight low-repetition resistance training; whereas, the *ACE* II and *ACTN3* XX genotypes demonstrated a greater improvement with low-load high-repetition resistance training. However, this study comprised a small sample size, which consequently increases the likelihood of type 1 error occurrence [81]. Furthermore, to the author's knowledge, the results presented by Jones and colleagues [80] have yet to be replicated in an independent

cohort. Therefore, further intervention studies are required in order to establish a strong evidence base, before practical recommendations can be proposed regarding the results of this review. However, once a strong evidence base has been established which supports genetic-based programme design, this review can be used alongside a number of other extensively evidenced/researched genotypes to form the basis of genetic-based training. Indeed, studies have also showcased that differences in biomarkers of muscle damage, hormones, and inflammatory responses, may be influenced by genotype variation [82,83]. Therefore, the utilisation of genetic information in the future may not only aid in optimising training adaptation, but also the planning of athlete workloads and recovery.

5 Conclusion

The results of this review provide further evidence that individual genetic variation likely contributes towards athlete status. Our findings suggest that genetic variation can differentiate athletes of different competitive playing statuses in a homogenous team-sport cohort. Specifically, this review has showcased that the R allele and RR genotype of the *ACTN3* R577X polymorphism are overrepresented in PRO football players; whereas the D allele and DD genotype of the *ACE* I/D polymorphism are overrepresented in youth players. These overrepresentations may be explained by the association of the *ACTN3* RR genotype and *ACE* DD genotype with power-orientated phenotypes and the relative contribution of these power-orientated phenotypes to success in football. As such, the *ACTN3* R577X and *ACE* I/D polymorphisms are likely (albeit relatively minor) contributing factors which influence athlete status in football.

Declarations

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Contributions All authors contributed to the conception of the article. ABTM drafted the article and performed the literature search and analysis. All authors contributed to interpretation of the results and critically revised the work. All authors read and approved the final manuscript.

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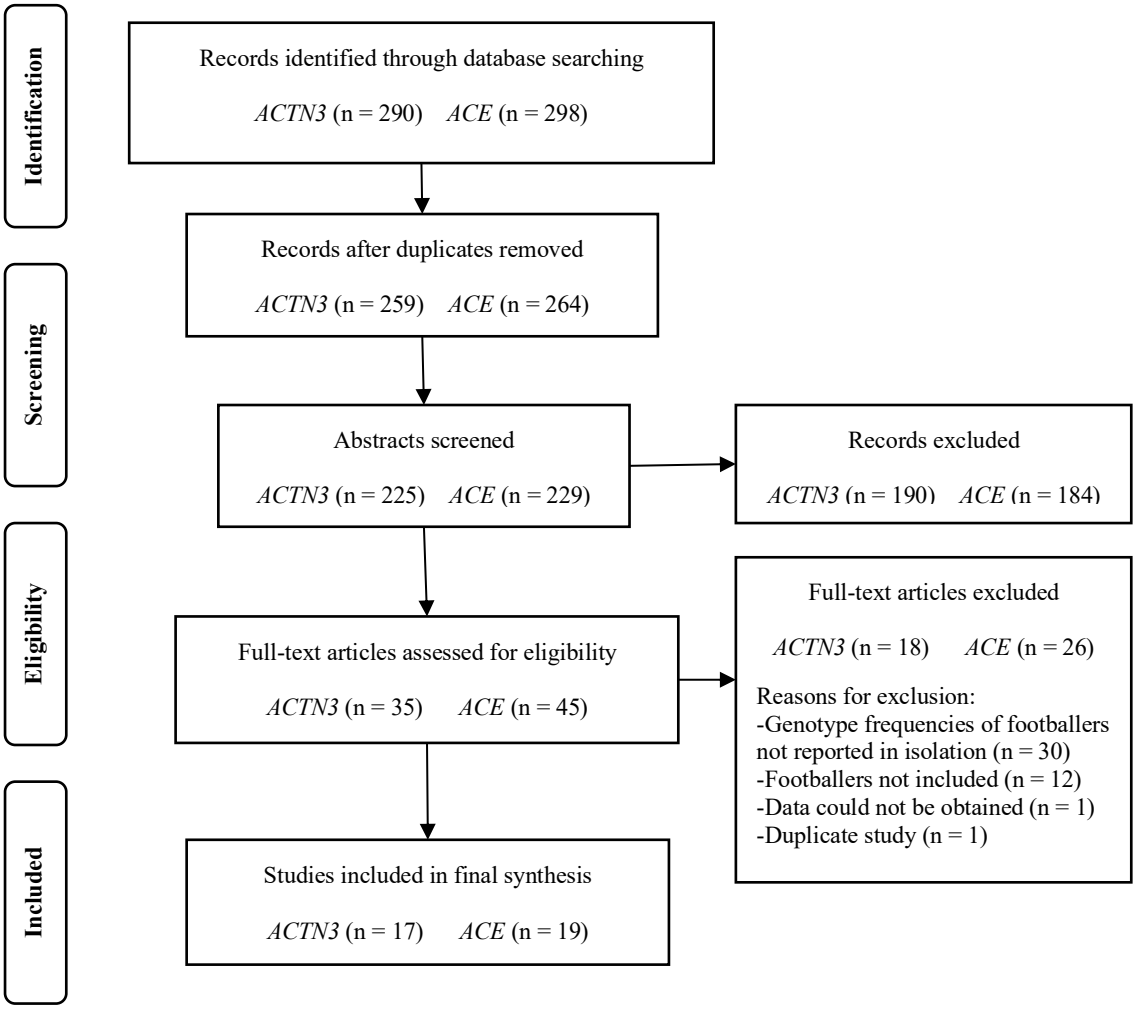

Figure 1. Flow diagram of systematic search process.

Table 1. Statistical analysis of all studies investigating the *ACTN3* R577X polymorphism (n = 15)

Model		Association test			Heterogeneity		Bias
		OR	**95% CI**	***P***	***P***	I^2	***Egger's***
Case-Control							
Allele contrast		1.30	1.15-1.46	<0.001	0.68	0.00	0.88
Recessive		1.42	1.20-1.68	<0.001	0.22	0.24	0.87
Dominant		1.35	1.06-1.72	0.016	0.33	0.13	0.97
Over-dominant		0.84	0.66-1.07	0.16	0.05	0.45	0.93
RR vs. XX		1.67	1.28-2.17	<0.001	0.63	0.00	0.98
RR vs. RX		1.38	1.07-1.78	0.013	0.08	0.41	0.76
RX vs. XX		1.17	0.90-1.51	0.23	0.11	0.38	0.92
Case-Control & Cohort							
Allele contrast		1.26	1.15-1.38	<0.001	0.72	0.00	0.48
Recessive		1.31	1.15-1.50	<0.001	0.23	0.18	0.89
Dominant		1.40	1.17-1.69	<0.001	0.49	0.00	0.64
Over-dominant		0.93	0.78-1.12	0.45	0.05	0.38	0.75
RR vs. XX		1.60	1.31-1.96	<0.001	0.72	0.00	0.62
RR vs. RX		1.23	1.02-1.49	0.029	0.08	0.34	0.83
RX vs. XX		1.29	1.06-1.57	0.010	0.18	0.24	0.69
PRO vs. NP							
Allele contrast	PRO	1.35	1.18-1.53	<0.001	0.77	0.00	0.34
	NP	1.07	0.90-1.27	0.44	0.90	0.00	0.30
Recessive	PRO	1.48	1.23-1.77	<0.001	0.30	0.14	0.70
	NP	1.00	0.78-1.29	0.97	0.74	0.00	0.46
Dominant	PRO	1.40	1.09-1.81	0.010	0.16	0.30	0.83
	NP	1.27	0.91-1.76	0.16	1.00	0.00	0.34
Over-dominant	PRO	0.84	0.63-1.12	0.24	0.020	0.51	0.89
	NP	1.13	0.89-1.43	0.33	0.79	0.00	0.66
RR vs. XX	PRO	1.77	1.34-2.34	<0.001	0.57	0.00	0.61
	NP	1.22	0.84-1.76	0.29	0.95	0.00	0.21
RR vs. RX	PRO	1.41	1.07-1.86	0.014	0.07	0.40	0.98
	NP	0.94	0.72-1.23	0.68	0.72	0.00	0.52
RX vs. XX	PRO	1.22	0.79-1.87	0.36	0.028	0.50	0.91
	NP	1.30	0.92-1.84	0.14	0.99	0.00	1.00
Caucasian & Brazilian							
Allele contrast	Caucasian	1.32	1.14-1.53	<0.001	0.39	0.05	0.73
	Brazilian	1.23	1.07-1.41	0.003	0.45	0.00	0.47
Recessive	Caucasian	1.41	1.14-1.74	0.001	0.11	0.42	0.67
	Brazilian	1.19	0.98-1.44	0.07	0.64	0.00	0.69
Dominant	Caucasian	1.49	1.11-2.00	0.008	0.79	0.00	0.82
	Brazilian	1.52	1.15-1.99	0.003	0.17	0.35	0.92
Over-dominant	Caucasian	0.91	0.75-1.12	0.38	0.11	0.42	0.69
	Brazilian	1.07	0.89-1.29	0.48	0.59	0.00	0.45
RR vs. XX	Caucasian	1.71	1.24-2.36	0.001	0.60	0.00	0.60
	Brazilian	1.61	1.19-2.16	0.002	0.23	0.27	0.94
RR vs. RX	Caucasian	1.36	0.96-1.92	0.08	0.08	0.47	0.61
	Brazilian	1.08	0.88-1.32	0.46	0.66	0.00	0.85
RX vs. XX	Caucasian	1.34	0.98-1.83	0.06	0.61	0.00	0.97
	Brazilian	1.48	1.10-1.98	0.009	0.17	0.35	0.98

Note. Allele contrast (R vs. X); Recessive (RR vs. RX+XX); Dominant (RR+RX vs. XX); Over-dominant (RX vs. RR+XX); OR = Odds Ratio; CI = Confidence Interval; PRO = Professional; NP = Non-Professional.

Table 2. Statistical analysis of all studies investigating the *ACE* I/D polymorphism (n = 17)

Model		Association test			Heterogeneity		Bias
		OR	**95% CI**	***P***	***P***	I^2	***Egger's***
Case-Control							
Allele contrast		0.88	0.73-1.07	0.21	0.001	0.61	0.16
Recessive		0.84	0.69-1.03	0.09	0.44	0.01	0.72
Dominant		0.84	0.62-1.15	0.28	<0.001	0.66	0.09
Over-dominant		0.93	0.74-1.16	0.51	0.040	0.44	0.04
II vs. DD		0.82	0.58-1.17	0.27	0.025	0.47	0.19
II vs. ID		0.98	0.79-1.21	0.82	0.65	0.00	0.27
ID vs. DD		0.84	0.62-1.14	0.26	0.002	0.60	0.08
Case-Control & Cohort							
Allele contrast		0.87	0.75-1.02	0.09	0.004	0.53	0.10
Recessive		0.86	0.72-1.03	0.10	0.69	0.00	0.69
Dominant		0.82	0.63-1.07	0.14	<0.001	0.62	0.05
Over-dominant		0.90	0.73-1.10	0.31	0.019	0.45	0.03
II vs. DD		0.79	0.60-1.05	0.10	0.08	0.34	0.12
II vs. ID		1.00	0.83-1.22	0.97	0.75	0.00	0.21
ID vs. DD		0.81	0.62-1.06	0.13	0.001	0.58	0.05
PRO vs. NP							
Allele contrast	PRO	1.01	0.86-1.19	0.88	0.96	0.00	0.67
	NP	0.85	0.73-0.99	0.044	0.15	0.35	0.45
Recessive	PRO	0.97	0.72-1.29	0.82	0.99	0.00	0.99
	NP	0.88	0.65-1.18	0.39	0.20	0.29	0.38
Dominant	PRO	1.05	0.82-1.34	0.71	0.86	0.00	0.73
	NP	0.78	0.61-0.98	0.032	0.25	0.22	0.44
Over-dominant	PRO	1.07	0.85-1.34	0.57	0.81	0.00	0.62
	NP	0.86	0.69-1.07	0.18	0.22	0.27	0.95
II vs. DD	PRO	1.05	0.74-1.49	0.77	0.99	0.00	0.95
	NP	0.77	0.55-1.08	0.14	0.22	0.26	0.26
II vs. ID	PRO	0.94	0.69-1.27	0.69	0.98	0.00	0.85
	NP	0.97	0.71-1.33	0.85	0.20	0.28	0.51
ID vs. DD	PRO	1.05	0.80-1.36	0.73	0.80	0.00	0.55
	NP	0.76	0.59-0.97	0.030	0.38	0.07	0.56
Caucasian & Brazilian							
Allele contrast	Caucasian	0.81	0.57-1.14	0.23	0.004	0.74	0.04
	Brazilian	0.85	0.70-1.03	0.09	0.37	0.07	0.63
Recessive	Caucasian	0.75	0.57-1.00	0.05	0.61	0.00	0.50
	Brazilian	0.83	0.60-1.14	0.25	0.25	0.26	0.07
Dominant	Caucasian	0.75	0.42-1.34	0.34	<0.001	0.82	0.03
	Brazilian	0.77	0.55-1.06	0.11	0.17	0.38	0.73
Over-dominant	Caucasian	0.83	0.53-1.29	0.40	0.009	0.70	0.08
	Brazilian	1.05	0.64-1.71	0.85	0.018	0.67	0.29
II vs. DD	Caucasian	0.65	0.38-1.10	0.11	0.08	0.52	0.08
	Brazilian	0.80	0.52-1.22	0.29	0.31	0.17	0.58
II vs. ID	Caucasian	1.05	0.77-1.44	0.75	0.59	0.00	0.28
	Brazilian	0.88	0.63-1.23	0.45	0.10	0.48	0.19
ID vs. DD	Caucasian	0.76	0.41-1.42	0.39	<0.001	0.82	0.07
	Brazilian	0.82	0.47-1.42	0.48	0.09	0.51	0.57

Note. Allele contrast (I vs. D); Recessive (II vs. ID+DD); Dominant (II+ID vs. DD); Over-dominant (ID vs. II+DD); OR = Odds Ratio; CI = Confidence Interval; PRO = Professional; NP = Non-Professional.

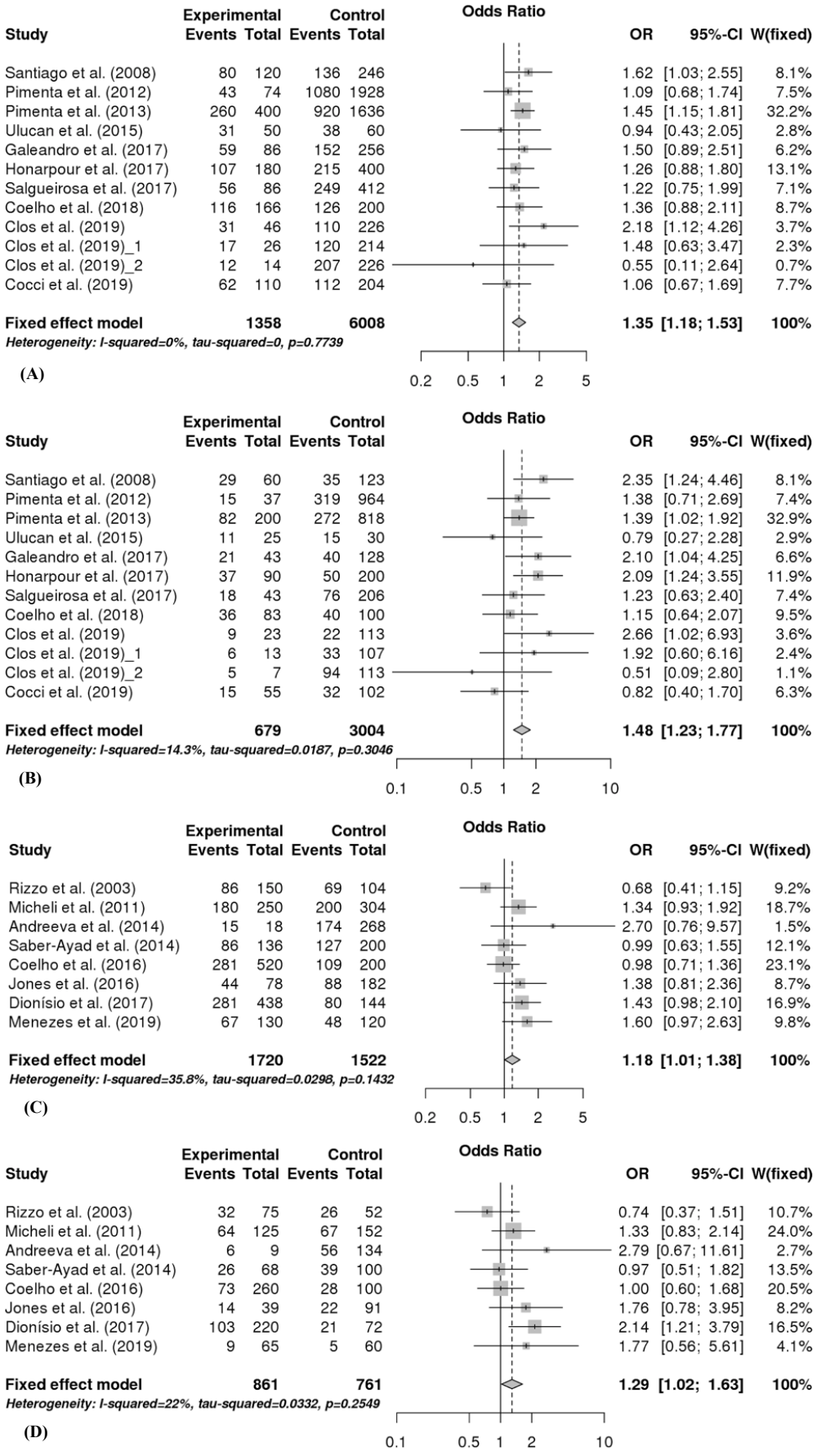

Figure 2. Forest plots of significant PRO and NP comparison models. (A) *ACTN3* R vs. X allele in PROs; (B) *ACTN3* RR vs. RX+XX in PROs; (C) *ACE* D vs. I allele in NPs; (D) *ACE* DD vs. DI+II in NPs.